Important factors to consider for acrylamide mitigation in potato crisps using pulsed electric fields

Highlights

- PEF was applied for the reduction of acrylamide content in potato crisps
- PEF pre-treatment was compared to the conventional blanching pre-treatment
- PEF protocol and sample preparation were optimized for industrial application
- The quality of PEF-treated potato crisps (colour and texture) was evaluated
- A reduction of 30% of acrylamide content was achieved after applying PEF

Industrial relevance

The Commission Regulation (EU) 2017/2158 of 20 November 2017 has introduced new benchmark levels and mitigation strategies for the reduction of the presence of acrylamide in foods, directing food businesses to the research of measures to lower the acrylamide formation in foods. The actual industrial production process of fried potato crisps involves the use of many mitigation strategies, such as a blanching of raw potatoes. However, the traditional blanching treatment presents several practical drawbacks and leads to undesirable changes of the product quality. The application of PEF as a pre-treatment could reduce the acrylamide content in deep-fat fried potato crisps. This preliminary study gives important indications regarding the possibility of combining a PEF pre-treatment on raw potato slices with subsequent industrial processing steps for the production of potato crisps with low acrylamide concentration.

Important factors to consider for acrylamide mitigation in potato crisps using pulsed electric fields

Jessica Genovese[a]*, Silvia Tappi[b], Wei Luo[a], Urszula Tylewicz[a], Silvia Marzocchi[a], Sara Marziali[c], Santina Romani[a,b], Luigi Ragni[a,b], Pietro Rocculi[a,b]

[a]Department of Agricultural and Food Sciences, *Alma Mater Studiorum*, University of Bologna, Campus of Food Science, Piazza Goidanich 60, Cesena (FC), Italy

[b]Interdepartmental Centre for Agri-Food Industrial Research, *Alma Mater Studiorum*, University of Bologna, Campus of Food Science, Via Quinto Bucci 336, Cesena (FC), Italy

[c]Department of Agricultural, Environmental and Food Sciences, University of Molise, Via De Sanctis snc, Campobasso, Italy

*Corresponding author

Email: jessica.genovese3@unibo.it

Abstract

This preliminary study aimed to compare the application of pulsed electric field (PEF) with a traditional blanching as pre-treatments before frying for the mitigation of acrylamide content in potato crisps.

Measuring the degree of cell disintegration index (p_o) and the changes in water electrical conductivity during washing of potato slices, PEF protocol and sample preparation scheme were optimized. Peeled potato slices (thickness 1.5 ± 0.2 mm) were subjected to PEF (1.5 kV cm^{-1}, pulse duration 10 µs, total treatment time 10 ms, pulse frequency 100 Hz) and to blanching (85°C for 3.5 min) pre-treatments and then to washing in water, evaluating the reduction of acrylamide precursors (reducing sugars and free asparagine). After frying (175°C, 3 min), product quality, in terms of colour, texture and acrylamide content were evaluated. Results showed that PEF promoted acrylamide precursors leaching followed by a reduction of the final acrylamide content of around 30%, significantly higher if compared to the reduction obtained with blanching, with only slight modifications of the final quality of the product, in terms of colour and texture.

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regarding the possibility of combining a PEF pre-treatment on raw potato slices with subsequent industrial processing steps for the production of potato crisps with low acrylamide concentration.

Keywords:
Potato crisps; Acrylamide; electroporation; mass transfer; colour, texture

1. Introduction

Acrylamide has been identified as a contaminant in a range of fried and oven-cooked foods (e.g. French fries, potato crisps, bread and cereal) and drinks (e.g. coffee); and its classification as probably carcinogenic in humans has caused worldwide concerns (International Agency for Research on Cancer, 2014). Although most epidemiologic studies examining the relationship between estimated dietary consumption of acrylamide and specific cancer resulted inconclusive, experimental animal studies identified neurotoxicity, carcinogenicity, adverse effects on male reproduction, as possible critical endpoints for acrylamide toxicity (European Food Safety Authority, 2015).

Certain foods, more specifically certain food components such as asparagine and reducing sugars, could lead to the formation of acrylamide during heat treatment at temperatures above 120 °C as a result of the Maillard reaction (Mottram, Wedzicha, & Dodson, 2002). Among fried carbohydrate-rich foods, potato crisps contribute to a substantial proportion of the estimated intake of acrylamide in the European adult population (European Food Safety Authority, 2015). Statistical data revealed that in Europe the consumption of salty snacks, in particular potato chips/crisps, stands to an average of 1.5 kg per capita in 2018 (Statista, 2018).

National authorities, together with research institutes and food industries reported many strategies of controlling and minimizing the formation of acrylamide, with particular concern to fried potato-based products, due to the presence of large concentration of acrylamide precursors in the potato. In fact, levels up to 4000 ppb of this contaminant have been detected in potato crisps (Becalski, Lau, Lewis, & Seaman, 2003).

Recently, the Commission Regulation (EU) 2017/2158 of 20 November 2017 has established new "mitigation measures and benchmark levels for the reduction of the presence of acrylamide in foods", which aim to ensure that food businesses put in place steps to mitigate acrylamide formation (European Union, 2017). Powers, Mottram, Curtis, & Halford (2017) analysed European manufacturers' data on acrylamide in potato crisps from 2002 to 2016, and the study showed that, even though acrylamide levels in potato crisps in Europe have been levelled off in recent years, more than 5% of samples exceeded the regulated benchmark level for potato crisps (750 ng g^{-1}).

Several investigations have proposed mitigation ways to be applied at different stages of the manufacturing process of potato crisps, in order to reduce the concentration of acrylamide precursors. Among conventional mitigation strategies, hot water blanching of potato slices appeared to facilitate the extraction of Maillard reaction substrates, in addition to enzyme inactivation, also improving the colour uniformity and texture, and oil absorption reduction (Pedreschi, Kaack, & Granby, 2004; Mestdagh et al., 2008). However, this thermal

pre-treatment presents the drawbacks of being time-intensive, high energy-consuming and of promoting considerable modifications of sensorial properties of final product.

Recent studies have proposed alternative non-thermal technologies in food processing and preservation. Pulsed electric fields (PEF) have been widely described as one of the most promising non-thermal novel technologies in the last decades, stimulating intensive research in several fields such as biotechnology, medicine and food processing. PEF treatment is based on the application of an external electric field applying short and intensive electric pulses. The application of PEF for potato snacks pre-treatment has been extensively studied, and many researchers have already reported high numbers of benefits that could be achieved by applying electric pulses to raw potatoes. Fauster et al. (2018) have recently described the impact of PEF treatment on potato structure and various potential advantages on quality and economic aspects of industrial French fries production, including reductions of cutting force, starch loss and oil uptake. Furthermore, the application of pulsed electric field treatments above a specific critical value of field strength is well known to enhance mass transfer from plant tissues, increasing the cell membrane permeabilization (Donsì, Ferrari, & Pataro, 2010). Following this principle, Jaeger, Janositz, & Knorr (2010) have stated that PEF treatment of raw potatoes could assist and increase the release of sugars and amino acids that represent the main substrates for the Maillard reaction, consequently leading to formation of lower amounts of acrylamide. Moreover, Janositz, Noack, & Knorr (2011) have reported a significant increase in the release of reducing sugars (fructose, glucose) and sucrose in potato slices after PEF application.

However, the effective reduction of acrylamide content in deep-fat fried potato products is still unclear, although many researchers observed a significant increase of acrylamide precursors extractability on PEF treated potatoes, reducing browning during frying (Ignat, Manzocco, Brunton, Nicoli, & Lyng, 2015). Besides, lab-scale investigations followed different trials' schemes, applying PEF as a pre-treatment of whole potato tubers before or after peeling, concentrating on the treatment itself, with low attention to the combination of other process operational units that could influence the outcome. It is necessary to understand how the rate of mass transfer promoted by applying certain electric field strengths could be influenced by other processes operations, and how the quality of the final product will be preserved.

This preliminary study aimed to evaluate the effect of the application of PEF as a pre-treatment, and the effect of time modulation of subsequent washing steps of treated potato slices, on the Maillard reaction substrates and final acrylamide content, and on the quality of fried potato crisps. Measuring the degree of cell disintegration index (p_o) (Angersbach, Heinz, & Knorr, 1999) and the changes in water electrical conductivity during washing of potato slices, it is possible to optimise either PEF protocol and sample preparation scheme, in order to maximise the release of acrylamide precursors from the raw tissue. The efficiency of PEF as a non-thermal pre-treatment on the acrylamide reduction was compared to a conventional blanching in hot water usually used as a pre-treatment in the fried potato industrial lines.

2. Materials and Methods

2.1 Sampling

Potato tubers (*Solanum tuberosum L.*) of the Lady Claire variety (suitable for industrial processing), were purchased at a local market one month after harvesting and stored in the dark at 10 ± 2 °C for a maximum of two weeks before trials. The storage temperature was chosen according to Pinhero et al. (2009). The initial moisture content of potato tubers was 81.92 ± 0.58 %, evaluated by drying 5g of fresh potato tissue in a convection oven at 105 °C until a constant weight was achieved. Before pre-treatment, only tubers of a similar size and shape were selected, manually peeled and sliced (1.5 ± 0.2 mm in thickness) using a stainless-steel electric slicer machine (Mod. KAFPL0922N CAD, Italy). Both whole tubers and slices were rinsed for 1 min in tap water (water temperature: 18 ± 2 °C) and subsequently submitted to blanching or PEF treatment, followed by washing as better detailed in section 2.4.

2.2 Pre-treatments

2.2.1 Blanching

Blanching was performed by immersing potato slices in hot distilled water at 85 °C and for 3.5 min stirring with a product-to-water ratio of around 1:2 (w/w), according to the method used by Pedreschi et al. (2011). The use of distilled water, according to the same authors, allowed to maximise the diffusion of acrylamide precursors from potato tissue. Hence, although the use of distilled water is not industrially relevant, in this study distilled water blanching was chosen in order to compare pulsed electric fields achievements with the best performances of blanching.

2.2.2 Pulsed electric fields (PEF) treatment

PEF pre-treatments were performed using a lab-scale PEF unit delivering a maximum output current and voltage of 60 A and 8 kV, respectively (Mod. S-P7500, Alintel, Italy). The generator provides monopolar rectangular-shape pulses and adjustable pulse duration (5-20 μs), pulse frequency (50-500 Hz) and total treatment time (1-600 s). The treatment chamber (50 mm length x 50 mm width x 50 mm height) consisted in two parallel stainless-steel electrodes (3 mm thick) with a 47 mm fixed gap. Output voltage and current were monitored using a PC-oscilloscope (Picoscope 2204a, Pico Technology, UK). Samples were treated at room temperature in tap water, with an initial electrical conductivity of 542 ± 2 μS cm^{-1} at 25 °C, measured using an EC-meter (Mod. Basic 30, Crison, Spain). Trials were conducted delivering n = 1000 pulses at fixed pulse width (10 ± 1 μs), frequency (100 Hz) and repetition time (10 ± 1 ms). An electric field strength of 1.5 kV cm^{-1} was selected in order to achieve irreversible electroporation (Faridnia et al., 2015). Temperature changes due to PEF treatments were negligible.

Fig. 1 shows a schematic diagram of the PEF apparatus used. The same PEF protocol was applied on both whole tubers (Fig.1 A) and potato slices (Fig.1 B), in order to understand if the different exposed surface-to-volume ratio could have affected the efficiency of mass transfer. In both cases, the treatment chamber was filled with a product–to–water ratio of around 1:5 (w/w).

146

147 2.3 Determination of cell disintegration index (p_o)

148 Cell disintegration index (p_o) was analysed, according to Angersbach, Heinz, & Knorr (1999), for PEF
149 treated whole tubers and slices. The method is based on changes in the electrical properties of an intact and
150 permeabilized biologic membrane (considered equal to a resistor and a capacitor). The electrical conductivity
151 (σ) for intact and processed samples was obtained by impedance measurements at low (1 kHz) and high (5
152 MHz) frequencies. The impedance spectra were acquired using a precision impedance meter (Mod. LCR-
153 8105G, GW Instek, Taiwan) connected to a parallel plate measuring cell with adjustable gap. The cell
154 disintagration index (p_o) was calculated by the following equation:

155

156 $$p_o = \frac{\left(\sigma_h^i/\sigma_h^s\right)\sigma_l^s - \sigma_l^i}{\sigma_h^i - \sigma_l^i} \quad (1)$$

157

158 where σ_l^i and σ_l^s indicate the electrical conductivity of untreated and treated cell material at low frequency (1
159 kHz), respectively; and σ_h^i and σ_h^s indicate the electrical conductivity of untreated and treated material at high
160 frequency (5 MHz), respectively. The parameter p_o ranges from 0 for intact tissue to 1 for complete disrupted
161 tissue.

162

163 2.4 Washing in water

164 Potato slices, either untreated or subjected to PEF or blanching were soaked and stirred in tap water (18
165 ± 2 °C), with an initial electrical conductivity of 319 ± 4 μS cm^{-1} at 25 °C, and with a product-to-water ratio
166 around 1:1.5 (w/w). In order to select the optimal soaking time as a result of maximum release of intracellular
167 compounds into the external aqueous phase due to PEF-induced electroporation, washing times of 5, 10, 15,
168 20 min were tested on PEF-processed potato slices before frying. For each dipping time, changes in water
169 electrical conductivity were registered using an EC-meter (Mod. Basic 30, Crison, Spain). The selected
170 washing times, based on the highest water electrical conductivity variation recorded, were applied also to
171 untreated (control) and blanched potato slices before frying, in order to obtain comparable results for further
172 analysis.

173

174 2.5 Frying conditions

175 Untreated (control), blanched and PEF-treated potato slices were deep-fried in high-oleic sunflower oil
176 using an electrical fryer (Mod. MFR280R, Fama Industrie, Italy) at 175 °C as initial oil temperature. Potato-
177 to-oil weight ratio was around 1:10 and slices were fried for 3 min until a final moisture of ~2% (wet basis)
178 was reached. Temperatures of frying oil and frying potatoes were monitored using thermocouples sensors type
179 K connected to a data logging system (Mod. 9211A, National Instruments™, Texas).

180

2.6 Acrylamide precursors in raw potatoes

2.6.1 Reducing sugars analysis

The concentrations of glucose and fructose in raw potatoes were quantified using the method described by Rodriguez-Saona et al. (1997) with few modifications. A 2g sample of freeze-dried potato slices was dissolved in 20 ml of distilled water using ultrasonic bath (Elmasonic, Germany). The sample was centrifuged (Centrifuge Thermo Electron, USA) 15 minutes at 3500 rpm, and the supernatant was collected. A 1 ml aliquot of the solution was passed through C18 cartridges (1000mg, 6mL; Phenomenex) for purification. Subsequently, the sample was resuspended in 0.25 ml of deionised distilled water before injection into HPLC. Glucose and fructose were determined with HPLC Agilent Infinity 1260 (Agilent Technologies, Santa Clara, CA, USA) coupled to ELSD PL-ELS 1000 (Agilent, Santa Clara, CA, USA) as detector. The analytical column was a SphereClone NH2 (250 mm x 4.60 mm i.d.; 5 μm particle size) (Phenomenex, Torrance, CA, USA); the elution was in isocratic mode using a mixture of water:acetonitrile 70:30 (v/v) as mobile phase at a flow rate of 0.6 mL/min. The sample injection volume was 10 μL. All samples were analysed in triplicate.

2.6.2 Asparagine analysis

The concentration of free asparagine in raw potatoes was quantified dissolving 5g sample of freeze-dried potato slices in 50 ml of distilled water using ultrasonic bath (Elmasonic, Germany) for 10 min. The sample was centrifuged (Centrifuge Thermo Electron, USA) 15 minutes at 3500 rpm, the supernatant collected, micro-filtered (0.22 μm) before HPLC analysis. Free asparagine was determined with HPLC Agilent Infinity 1260 (Agilent Technologies, Santa Clara, CA, USA) coupled to a diode array detector (UV wavelength set at 338 and 262 nm). In order to obtain the derivatization sample, a 200 μL sample was added to 400 μL borate buffer with 50 μL o-phtalaldehyde-3-mercaptopropionic acid (OPA) reagent. Chromatographic conditions are described in Plata-Guerrero et al. (2009). All samples were analysed in triplicate.

2.7 Analysis of fried potato crisps

2.7.1 Computer Vision System (CVS) for colour determination

The surface colour of potato crisps was measured using a Computer Vision System (CVS) consisting of an illumination source, a colour digital camera (CDC), and an image processing software. Potato crisps samples were placed inside a dark box to exclude external light, and RGB images were acquired by a CDC (Mod. D7000, Nikon, Japan) with a 105 mm lens (Mod. AF-S Micro Nikkor), located vertically over the sample at a distance of 35 cm and connected to a PC. The lighting system consisted of four daylight fluorescent lamps (60 cm in length) connected to an electronic ballast to ensure uniform illumination, with a colour temperature of 6500 K and sited at an angle of 45° with the CDC. For each sample, untreated, blanched and PEF treated, 12 images were captured, each of one side of potato crisps. The pre-processing of RGB images, segmentation and colour quantification were performed with ImageJ analysis software (NIH, USA). The

218 average value of the segmented pixels in the CIE L^* a^* b^* colour space was registered as the colour of the
219 sample. From numerical values of a^* (green/red) and b^* (yellow/blue) chromatic parameters, hue angle (h°)
220 was calculated by the following equation and used to describe colour variations between samples:
221
222 $$h° = tan^{-1}(b^*/a^*) \quad (2)$$
223
224 2.7.2 Texture
225 Texture analysis of crisps were performed at room temperature (~20 °C) using a Texture Analyser TA-
226 XT2 (Mod. HDi 500, Stable Micro System, Surrey, UK) equipped with a 5 kg load cell. A puncture test was
227 selected to evaluate samples firmness and crispness. Crisp samples selected on the basis of uniform size and
228 shape, were placed on a support rig (HDP/CFS, Crisp Fracture Support Rig and corresponding Heavy Duty
229 Platform) and compressed for 3 mm distance using a spherical probe (P/0.25S) of ¼ - inch diameter (Salvador,
230 Varela, Sanz, & Fiszman, 2009). Force *vs* distance curves were obtained using a test speed of 1.0 mm s^{-1} and
231 the results obtained from 12 slices for each sample were expressed as firmness, calculated by means of
232 maximum force values and as crispness, calculated from means of linear distance (the length of a line joining
233 all fracture points in the force-deformation curve) between the first and the last facture peaks registered.
234
235 2.7.3 Acrylamide
236 *Sample extraction and SPE purification*. Potato chips samples were finely ground before the extraction;
237 1 g of sample was weighted into a polypropylene conical tube, and 100 µL 10µg/mL internal standard solution
238 ($^{13}C_3$-labelled acrylamide in MeOH) followed by 10 mL 0.1% (v/v) formic acid were added. After mixing with
239 Vortex for 10 min, the extract was centrifuged at 4500 rpm for 15 min. A 2 mL portion of clarified solution
240 was removed, avoiding collection of top oil layer when present, and filtered through paper filter. A solid-phase
241 extraction (SPE) was performed using C18 cartridges (1000mg, 6mL; Phenomenex). Cartridges were first
242 conditioned with 5 mL methanol followed by 5 mL water; 1 mL of filtered sample was loaded and washed
243 with 1 mL water. Eluition was performed with 1 mL acetone. Acetone was removed under nitrogen flow and
244 sample was dissolved in 1 mL 0.1% (v/v) formic acid before injection. SPE clean-up was performed on 3
245 extracts for each sample.
246 *HPLC-ESI-MS/MS analysis.* LC-ESI-MS/MS in positive ion mode (ESI^+) analyses were performed by an
247 Agilent 6420 triple quadrupole (Agilent, Santa Clara, United States) coupled to an Agilent 1290 Infinity LC
248 Pump equipped with an autosampler and a thermostated column oven, according to Calbiani, Careri, Elviri,
249 Mangia, & Zagnoni (2004) with some modifications. The analytical column was a Poroshell 120 C18, 3.0 x
250 100 mm, 2.7 µm (Agilent, USA) maintained at 20 °C. The elution was in isocratic mode using a mixture of
251 0.1% (v/v) aqueous formic acid and methanol (99.5/0.5, v/v) as mobile phase at a flow rate of 0.3 mL/min.
252 The sample injection volume was 10 µL. Full-scan analyses were performed in the 40-100 Da mass range,
253 acquiring the following transitions: extracted ion at m/z 55, due to the transition 72 > 55, and at m/z 58, due
254 to the transition 75 > 58 were used for the quantitative analysis. A calibration curve was made for the

quantification diluting stock solution of acrylamide with water in the 1.5-200 µg/L range. For acquisition and processing data, the Agilent MassHunter Workstation software was used.

2.8 Statistical analysis

Significant differences between results were calculated by paired samples Student's t-test, parametric analysis of variance (ANOVA) and Tukey multiple comparison, with a significance level of 95% ($p < 0.05$). If Shapiro-Wilk test for normality and Levene's test for homoscedasticity of data resulted statistically significant ($p < 0.05$), non-parametric multiple range test Kruskal-Wallis and Holm stepwise adjustment were used, with a significant level of 95% ($p < 0.05$) (R Foundation for Statistical Computing, Austria). All treatments were conducted in triplicate and results were expressed as mean ± standard deviations of replications.

3 Results and discussion

3.1 Experimental design set up

3.1.1 Effect of PEF treatment on cell disintegration index and degree of mass transfer

As reported by Angersbach et al. (1999), impedance measurements of plant tissues allow the evaluation of cell membrane permeabilization after applying PEF treatments.

To understand the efficiency of selected PEF protocol on the degree of cell disruption, and so on the level of mass transfer, cell disintegration index of PEF-treated potato tubers and potato slices was calculated from Eq. 1. Results are shown in Fig. 2; as expected, higher surface dimension exposed to electric pulses, being related to slices, resulted in higher cell disintegration, explaining the better efficiency of the pre-treatment if applied after the slicing step of the experimental scheme.

It is well established that measurements of the changes in electrophysical properties of untreated and treated cell tissues represent a reliable method to correlate PEF processing protocol and the cell damage degree in biological systems. Moreover, it has been widely reported that PEF-induced membrane permeabilization has the potential to effectively enhance mass transfer from the inner part of biological tissues, increasing the diffusion of cell compounds/metabolites (Donsì et al., 2010).

Another useful method to assess the intactness of cell membranes, reported by many researchers, is the measurement of ions/small molecules leakage from intracellular compartments. Washing of potato slices is a common practice in potato crisps production and allows to remove any surface starch residue prior to frying. In this work, different washing times of PEF-treated potato slices were evaluated, measuring the changes in electrical conductivity of residual washing water. Results are shown in Fig. 3; the maximum water conductivity variation was achieved after 5 min of washing, highlighting the period of time subsequent to PEF-treatment that permitted the highest release of cell fluid into the aqueous media.

Faridnia et al. (2015) reported that by suspending a PEF-treated potato tuber into an isotonic solution of mannitol (0.2 M) it was possible to monitor the electrolytes leakage from cell tissue by measuring changes in electrical conductivity of the surrounding media. Furthermore, it has been demonstrated that cell fluid leakage due to electroporation is function of time, as many transition processes induced by PEF (e.g. moisture and air redistribution among microscopic extracellular channels, mass transfer, partially or completely resealing of cell membranes) could last from seconds to hours (Oey et al., 2017). On the basis of this concept, it is clear that subsequent unit operations for potato crisps manufacturing need to be assessed in order to maximise mass transfers.

Thanks to the aforementioned introductory studies, in this work the experimental plan for the production of lab-scale deep-fat fried potato crisps was designed applying the different pre-treatments on raw potatoes directly after slicing and by selecting 5 min as the preferred time for potato slices washing step. A scheme with the experimental processing steps is shown in Fig. 4.

3.2 Deep-fat fried potato crisps analysis

3.2.1 Colour

Colour is one of the most important parameters to control during frying, being strongly related to consumer perception (Scanlon, Roller, Mazza, & Pritchard, 1994). Visual quality is associated with physical, chemical and sensorial evaluation and it is an important driver for buying being associated by consumers with flavour, safety, storage time, nutritional aspects and taste (Pedreschi, Kaack, & Granby, 2006a).

Colour of potato crisps is often measured using a colorimeter in L*a*b* units. According to Pedreschi, León, Mery, & Moyano (2006b), the use of a computer vision system (CVS) technique instead of the conventional colorimeter for monitoring the development of colour in potato crisps has different advantages, such as the possibility to analyse the whole surface of the product and to identify the presence of brown spots and other defects.

Fig. 5 shows images of control (5A), blanched (5B) and PEF treated (5C) potato crisps after frying. After blanching and PEF treatment, changes are noticeable in the appearance of potato slices compared to the control. The slices pre-treated by PEF showed a more uniform and lighter surface colour. Images have been converted from RGB into L*a*b* channels. The calculated values of L* and h° (Eq. 2) of the three samples are reported in Fig. 6.

Development of colour during frying is the result of the Maillard reaction, that involves reducing sugars and the amino acid asparagine and has been related to the formation of toxic compounds such as acrylamide. The extent of the Maillard reaction depends on the presence of reaction substrates and on frying parameters such as temperature and time (Romani et al. 2008; Romani et al. 2009).

The evolution of colour during frying is generally indicated by a decrease in L* and/or an increase of the redness parameter (a*) and of the hue (h°). Variation of colour parameters observed in the present study allowed the discrimination among samples. The three samples did not show significant differences in terms of

L*. On the other side, hue values increased for both pre-treated samples compared to the control, the highest values being related to the PEF pre-treated sample. Ignat et al. (2015) found similar results comparing the colour development during frying of blanched and PEF-treated potato slices. The increase of hue values testified a change from red to yellow colour, indicating the decrease of non-enzymatic browning reactions entity. Pre-frying treatments, such as blanching or dipping, are steps generally used in potato snacks manufacturing to stabilise the colour (Pedreschi et al., 2004). In fact, hot water blanching of raw potatoes could ease the leach out of superficial reducing sugars, main substrates or Maillard browning reactions. Moreover, the evolution of potato chips colour during frying has shown a good correlation (R^2 = 0.9569) with the acrylamide concentration, as reported by Pedreschi et al. (2005). Lighter and less red chips are related to a lower concentration of non-enzymatic browning reactions substrates and so to a lower content of acrylamide. The possibility of the release of the Maillard substrates from potato tissue treated with PEF has been already observed by various authors (Jaeger et al., 2010; Janositz et al., 2011). The increase of mass transfer upon PEF treatment is due to the effect of electroporation and cell permeabilization, that allow an increasingly diffusion of intracellular components across the membranes, as confirmed by the increase of conductivity of the washing water observed above.

3.2.2 Firmness & crispness

Texture is one of the main characteristics that influence the sensorial properties of potato-based products, and a delicate and crispy texture is recommended for potato crisps (Kita, 2014). Texture changes in potato crisps during frying result in the initial tissue softening and further crust development (Pedreschi, Moyano, Santis, & Pedreschi, 2007).

The changes of firmness and crispness of untreated, blanched and PEF treated potato crisps subjected to frying are shown in Fig. 7A and Fig. 7B, respectively. A slight, but significant reduction of both parameters was observed for blanched and PEF treated samples in comparison to the untreated one.

Structural changes of potato crisps could be influenced by many factors, e.g. firmness depends on the degree of starch gelatinization, on changes in the cell walls structure, mainly related to an increase in their permeability and on the reduction of intercellular adhesion between neighbouring cells (Moyano, Troncoso, & Pedreschi, 2007). Crispness instead is influenced mainly by dry matter content and oil uptake during frying (Abong, Okoth, Imungi, & Kabira, 2011).

Blanching at high temperature (80-100 °C), contrary to that at low temperature, has been already reported to promote potato tissue softening, by starch modification (hydration, swelling and gelatinization) along with β eliminative cleavage and pectin solubilization (Botero-Uribe, Fitzgerald, Gilbert, & Midgley, 2017). Moreover, high temperature blanching decreases polyphenol oxidase activity, responsible for enzymatic browning (Bingol et al., 2014).

Recently, some studies have been performed showing the effect of PEF pre-treatment on texture and other quality parameters of potato tissue before frying. Fauster et al. (2018) observed softening of the potato tissue and therefore the improvement of cutting behaviour (smoother surface and lower feathering). PEF treatment

(0.3- 1.2 kV/cm) caused also a significant softening of the ground tissues of sweet potato, resulted into lower force necessary for cutting (Liu et al., 2017). The softening of the tissue upon PEF treatment is probably due to the cell structure modification, mainly the increase in the membrane permeability and the irreversible cell breakdown, that in turn increase the water transfer, which is very important during potato-based product frying (Botero-Uribe et al., 2017). However, there is a lack of information in the literature about the effect of PEF pre-treatment on final products texture. Ignat et al. (2015) observed no differences in texture of PEF treated potato cubes (0.75 kV/cm and 2.50 kV/cm) in comparison to blanched and untreated ones. In our work, PEF-treated samples presented lower firmness and crispness in comparison to the untreated one, while no significant differences were observed between PEF-treated and blanched samples. The discrepancy could be due to the different shape of potato samples, indeed Ignat et al. (2015) performed their study on potato cubes (2 × 2 × 2 cm), while the present work was focused on 1.5 mm potato slices, as well as to different PEF process parameters and frying temperature.

3.2.3 Maillard reaction substrates and acrylamide content

As previously demonstrated, reducing sugars and asparagine represent the main limiting substrates of acrylamide formation in potato products (Amrein et al., 2003).

Table 1 shows the glucose, fructose and free asparagine content (mg kg^{-1}) in raw potato slices untreated and submitted to conventional and innovative pre-treatments (blanching and PEF, respectively), all followed by a washing step of 5 min.

Free asparagine was found at concentrations more abundant than reducing sugars, and its reduction in treated potato slices were higher than those found for glucose and fructose. In fact, PEF treatment allowed a reduction of 48% of free asparagine compared to the untreated sample, higher than the reduction reached by blanching (40%). Both pre-treatments, blanching and PEF, allowed just a slight reduction of the fructose initial content (4.9% and 5.4%, respectively). No glucose reduction was observed in PEF-treated potato slices; on the contrary the 27% of its reduction was shown in blanched potato samples.

It is well established that acrylamide precursors, reducing sugars and amino acids, are leached out by blanching treatment of raw potatoes (Zhang et al., 2018); on the other hand, PEF treatment has been mentioned as a potential alternative method to assist the removing of Maillard reaction substrates, and consequently reducing the acrylamide content in cooked potato-based products (Jaeger et al., 2010). While according to Janositz et al. (2011), PEF promoted a significant decrease in the reducing sugars content, the results of the present study seem to indicate that the main reduction is related to free asparagine.

The acrylamide content (mg kg^{-1}) of untreated, blanched and PEF-treated potato crisps is displayed in table 1. For the potato crisps pre-treated by PEF, the acrylamide content appeared lower compared with those pre-treated by conventional blanching. The PEF pre-treatment protocol and experimental conditions applied in this study resulted on a reduction of around 30% of acrylamide content compared to control (untreated), while only around the 17% of reduction was observed on blanched samples compared to control (untreated). Similar results of acrylamide reduction due to hot water blanching of potato slices were previously reported

by other authors (Pedreschi et al., 2011). The cell electroporation phenomenon induced by the application of the selected PEF protocol on raw potato slices resulted in a further improvement of the diffusion of Maillard reaction substrates and so of acrylamide reduction in fried potato crisps compared to the applied conventional pre-treatment.

4. Conclusions

Overall this preliminary study confirmed the high potentiality of the application of pulsed electric fields as a pre-treatment to improve the release of acrylamide precursors in raw potatoes and so to reduce the acrylamide content in deep-fat fried potato crisps. Moreover, important indications regarding the possibility of industrial application of PEF pre-treatment for the production of potato crisps were given. By monitoring PEF treatment parameters and other manufacturing steps, it was possible to achieve a consistent reduction of acrylamide due to its precursors leaching during the washing step, with only slight modifications of the final quality of the product, in terms of colour and texture.

Although PEF pre-treatment led to a significant reduction of acrylamide content in potato crisps if compared to untreated and blanched samples, the final amounts found were still higher than recommended legislative limits (0.75 mg kg^{-1}). In this direction other possible combined strategies need to be developed for industrial applicability. The combination of PEF and a mild blanching of raw potatoes and the monitoring of subsequent manufacturing operational units could enhance the extraction of reducing sugars and free asparagine and consequently the reduction of the acrylamide formation in potato crisps.

Figure captions

Fig. 1.

Schematic diagrams of experimental apparatus: (A) whole potato tuber PEF treatment; (B) potato slices PEF treatment.

Fig. 2.

Cell disintegration index (CDI) of PEF treated potato slices and tubers (E = 1.5 kV/cm; n = 1000). Results are expressed as means ± standard deviations (error bars) of n=20. Values with different letters differ significantly ($p < 0.05$).

Fig. 3.

Variations of water electrical conductivity affected by different washing times of PEF treated potato slices. Results are expressed as means ± standard deviations (error bars) of n=5. Values with different letters differ significantly ($p < 0.05$).

Fig. 4.

Scheme of experimental processing steps.

Fig. 5.

Examples of RGB images of untreated (a), blanched (b) and PEF-treated (c) potato crisps (left), and image conversion from RGB into L*a*b* channels (right). Pixels areas analysed are highlighted in yellow.

Fig. 6.

Lightness (A) and hue angle (B) of untreated, blanched and PEF-treated potato crisps. Results are expressed as means ± standard deviations (error bars) of n=20. Values with different letters differ significantly ($p < 0.05$).

Fig. 7.

Firmness (A) and crispness (B) of untreated, blanched and PEF-treated potato crisps. Results are expressed as means ± standard deviations (error bars) of n=20. Values with different letters differ significantly ($p < 0.05$).

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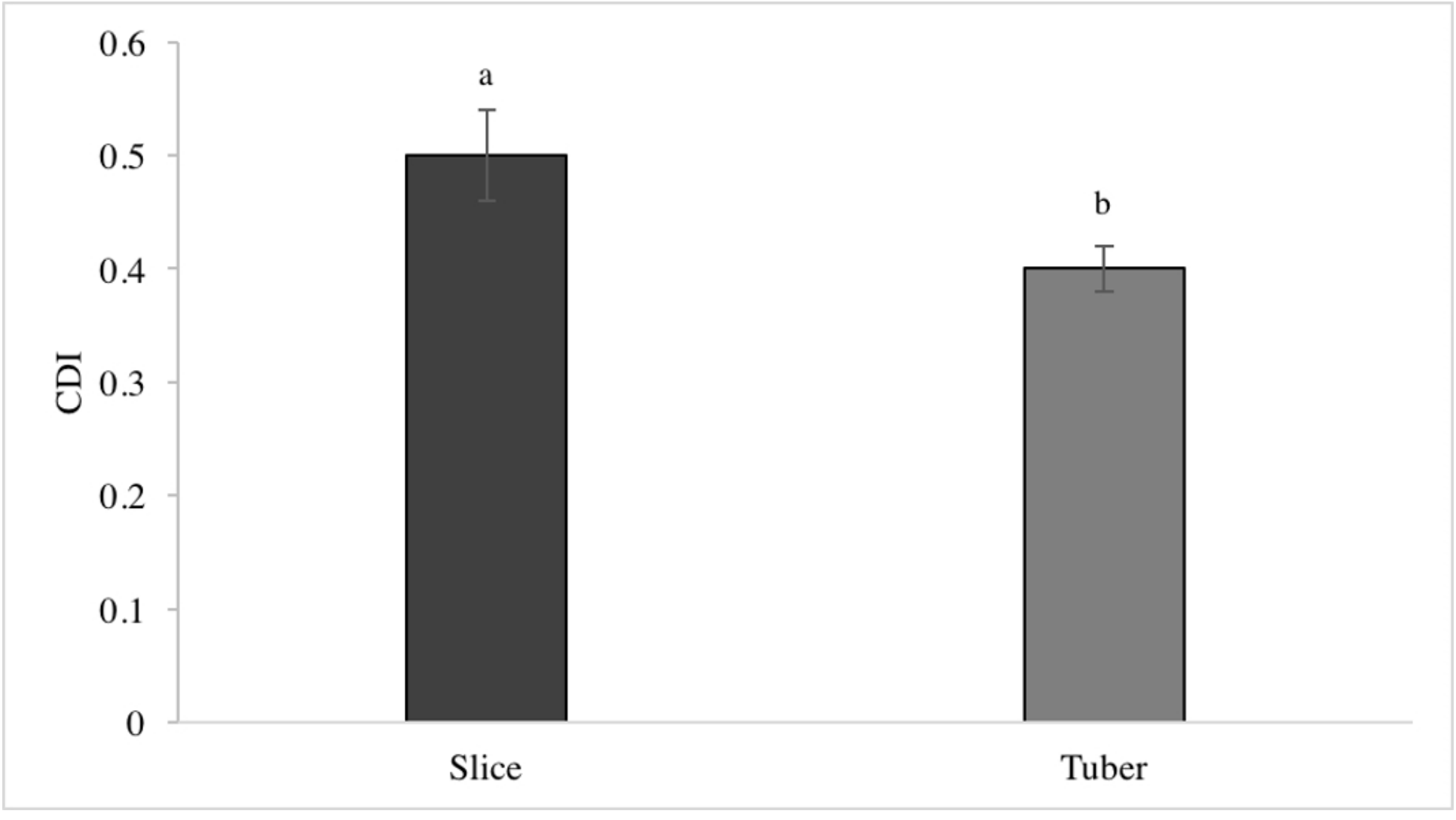
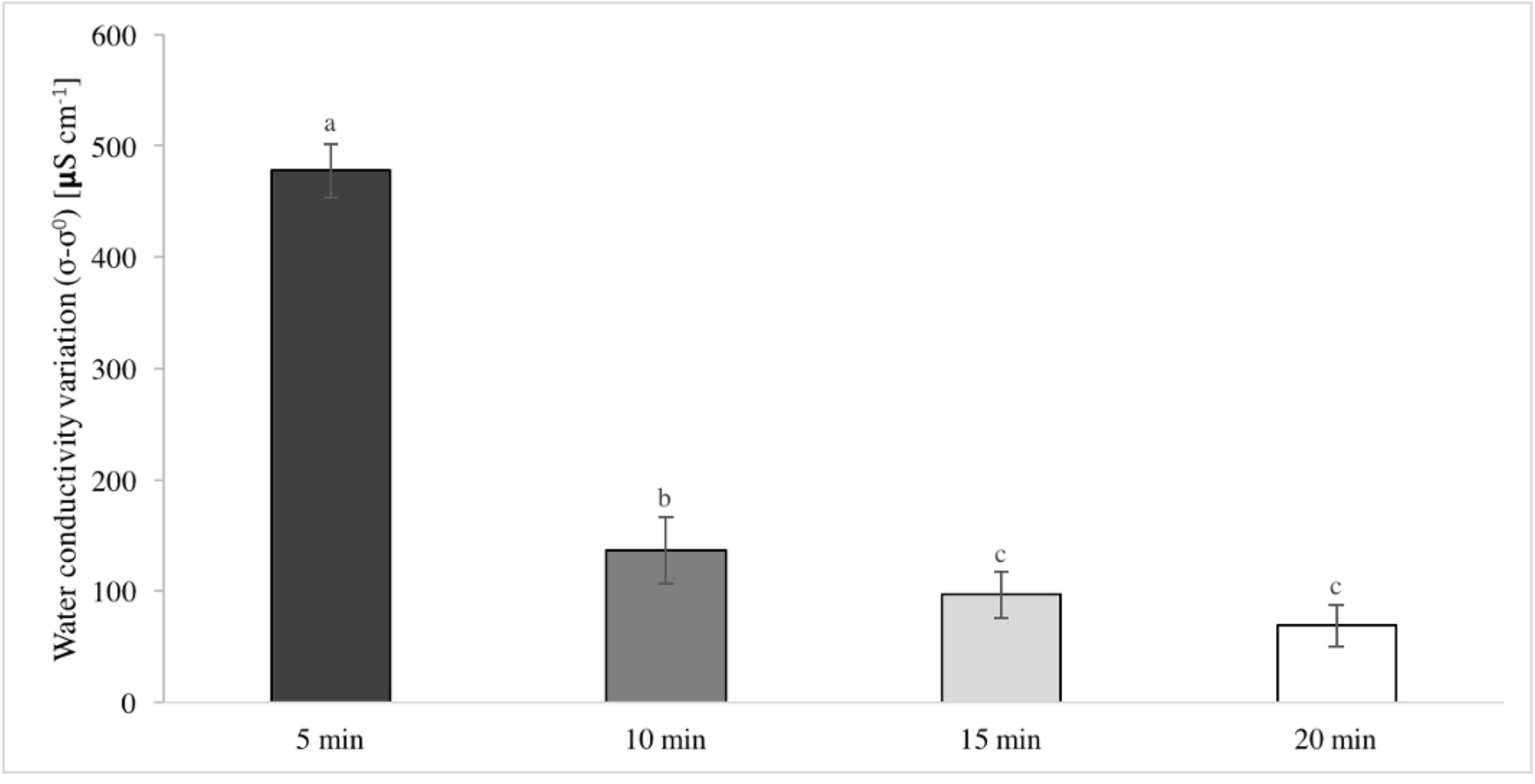
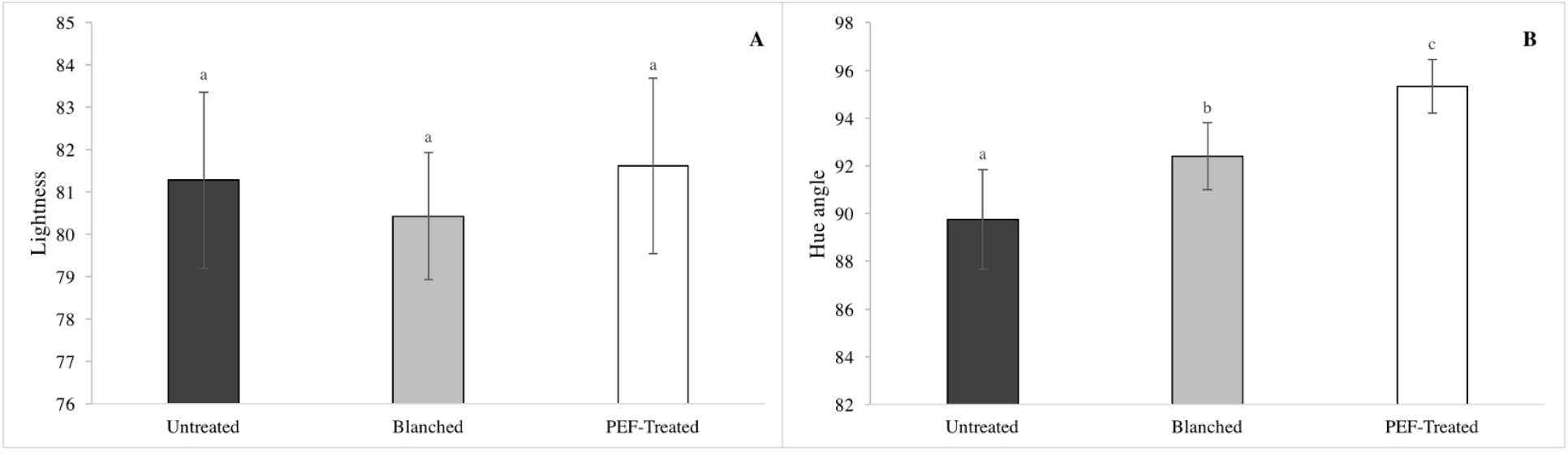
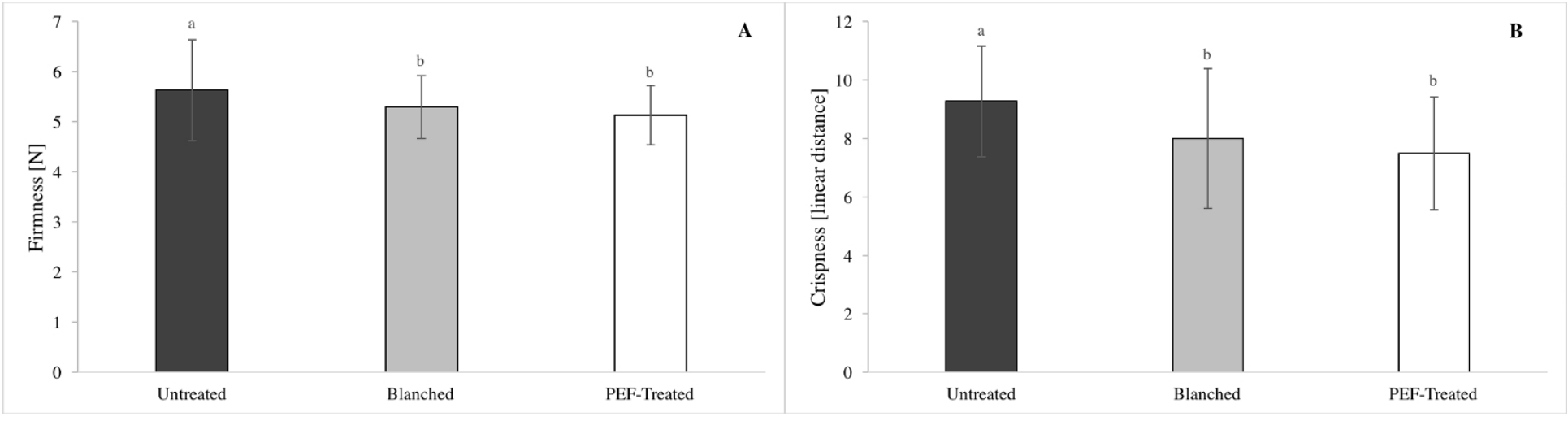

0.6
0.5
0.4
0.3
0.2
0.1
0
CDI
a
b
Slice
Tuber


Water conductivity variation (σ-σ⁰) [µS cm⁻¹]
600
500
400
300
200
100
0
a
b
c
c
5 min
10 min
15 min
20 min


A
Lightness
85
84
83
82
81
80
79
78
77
76
a
a
a
Untreated
Blanched
PEF-Treated
B
Hue angle
98
96
94
92
90
88
86
84
82
a
b
c
Untreated
Blanched
PEF-Treated


A
Firmness [N]
0
1
2
3
4
5
6
7
a
b
b
Untreated
Blanched
PEF-Treated
B
Crispness [linear distance]
0
2
4
6
8
10
12
a
b
b
Untreated
Blanched
PEF-Treated

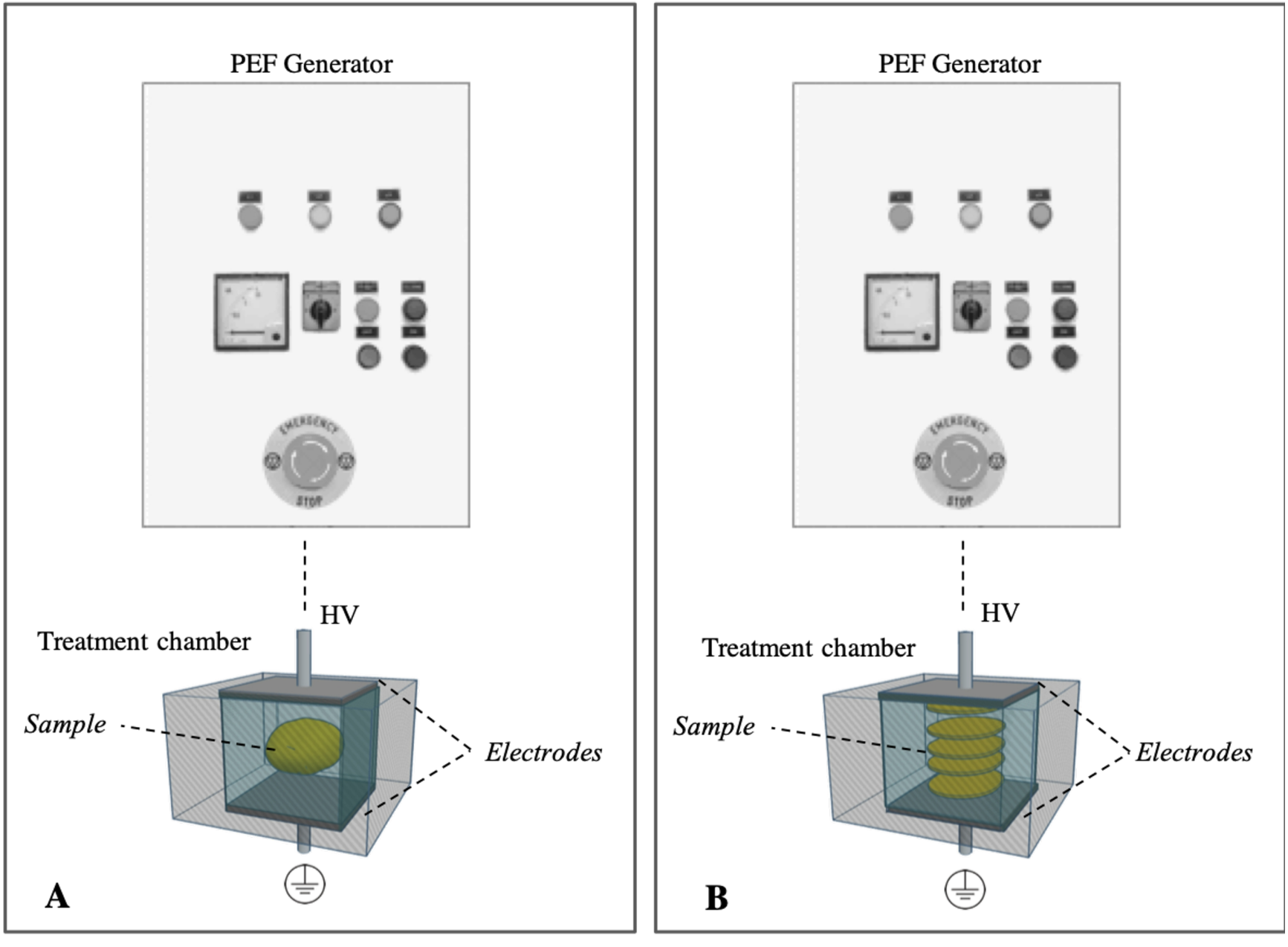

PEF Generator
HV
Treatment chamber
Sample
Electrodes
A
PEF Generator
HV
Treatment chamber
Sample
Electrodes
B

Table 1

Maillard substrates (glucose, fructose and free asparagine) contents of raw potato slices untreated, after blanching and after PEF treatment, and acrylamide content of untreated, blanched and PEF-treated potato crisps. Results are expressed in mg kg^{-1} of dry weight. Reduction percentages are calculated in relation to the untreated sample.

Sample	Glucose			Fructose			Asparagine			Acrylamide		
	Mean	%RSD*	Reduction (%)	Mean	%RSD	Reduction (%)	Mean	%RSD	Reduction (%)	Mean	%RSD	Reduction (%)
Untreated	70.0	14.1	-	58.9	1.7	-	10487.8	8.7	-	2.0	3.3	-
Blanched	50.9	11.9	27	56.1	3.5	4.9	6296.1	6.2	40	1.6	9.1	17
PEF-treated	75.4	10.6	n.a.**	55.8	3.1	5.4	5416.9	9.3	48	1.4	7.5	31

*percent relative standard deviation (n=3)
**not applicable